

# Derivation of Hill's Equation from Scale Invariance

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Received 2 April 2012; Revised 1 May 2012

## Abstract

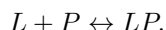
In biochemistry, the amount of the ligand that gets attached to macromolecule is often well described by an empirical formula with an empirical parameter  $n$ ; this formula is called Hill's equation. In this paper, we provide a theoretical derivation of this formula from the generalized equations of chemical kinetics. In the traditional chemical kinetics equations, the reaction rate is proportional to the product of the concentrations  $[c]$  of all reactants  $c$ . For example, for the reaction  $A + B \rightarrow C$ , the rate is proportional to the product  $[A] \cdot [B]$ ; for the reaction  $2A + B \rightarrow C$ , the rate is proportional to  $[A] \cdot [A] \cdot [B]$ , etc. These formulas explain specific cases of Hill's equation, corresponding to the case when  $n$  is an integer. To describe the general case, we assume a more general dependence  $f([A], [B])$  of the rate on the component concentrations. The numerical description of this dependence depends on the units that we use to describe the concentrations  $[A]$  and  $[B]$ . We make a natural assumption that this dependence is scale-invariant, in the sense that if we change units for measuring the concentrations  $[A]$  and  $[B]$ , the formula remains the same if we appropriately change the unit for measuring the rate. We show that this assumption leads to an explicit formula for the generalized chemical kinetics which, in turn, leads to an expression for the amount of the bound macromolecule, an expression that includes the Hill's equation as an important specific case. Thus, we get the desired theoretical derivation of the empirical Hill's equation.

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**Keywords:** Hill's equation, scale invariance, biochemistry, ligand

## 1 Formulation of the Problem

**Molecular binding in biochemistry.** Many biochemical reactions involve binding of a smaller molecule  $L$  (called *ligand*) to a large macromolecule  $P$ ; see, e.g., [1]:



Let us give a few examples:

- oxygen binds to haemoglobin: this is one of the most important biochemical reactions;
- acid content in the stomach regulated by histamine binding to histamine H2 receptor (special protein);
- human serum albumin, protein in human blood plasma, carries nutrients as ligands.

Since these reactions are important, it is desirable to predict the proportion of the bound macromolecules. This proportion can be determined as a ratio between the concentration  $[LP]$  of the bound macromolecule and the total concentration  $[P] + [LP]$  of free ( $P$ ) and bound ( $LP$ ) macromolecules:

$$\theta \stackrel{\text{def}}{=} \frac{[LP]}{[P] + [LP]}.$$

We want to find out how this proportion depends on the concentration  $[L]$  of the ligand.

**Hill's equation: description.** In many cases, the desired proportion  $\theta$  is described by a formula (called *Hill's equation*)

$$\theta = \frac{[L]^n}{K_d + [L]^n}.$$

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In this formula,  $K_d$  and  $n$  are empirical parameters.

The value  $n$  is different for different reactions. In some cases, we have  $n = 1$ . In many other cases, the value  $n$  is not an integer. For example, for the binding of oxygen to haemoglobin, we have  $n \approx 2.8$ .

**Chemical kinetics and the  $n = 1$  case of Hill's equation.** The quantitative results of chemical reactions are usually described by equations of chemical kinetics. In chemical kinetics, the reaction rate is proportional to the product of the concentrations  $[c]$  of all reactants  $c$ . For example, for the reaction  $A + B \rightarrow C$ , the reaction rate is proportional to the product  $[A] \cdot [B]$ :

$$\frac{d[A]}{dt} = -k \cdot [A] \cdot [B]; \quad \frac{d[B]}{dt} = -k \cdot [A] \cdot [B]; \quad \frac{d[C]}{dt} = k \cdot [A] \cdot [B].$$

For the reaction  $2A + B \rightarrow C$ , the reaction rate is proportional to  $[A] \cdot [A] \cdot [B]$ .

These formulas explain specific cases of Hill's equation, corresponding to the case when  $n$  is an integer. Indeed, in this case, we have two reactions:  $L + P \xrightleftharpoons{k_a} LP$  and  $LP \xrightleftharpoons{k_d} L + P$ . Thus, equilibrium is attained when

$$\frac{d[L]}{dt} = -k_a \cdot [L] \cdot [P] + k_d \cdot [LP] = 0.$$

So, at the equilibrium, we have  $k_a \cdot [L] \cdot [P] = k_d \cdot [LP]$ , and thus,

$$[LP] = \frac{k_a}{k_d} \cdot [L] \cdot [P].$$

Here

$$[P] + [LP] = \left(1 + \frac{k_a}{k_d} \cdot [L]\right) \cdot [P],$$

hence the desired ratio takes the form

$$\theta = \frac{[LP]}{[P] + [LP]} = \frac{\frac{k_a}{k_d} \cdot [L]}{1 + \frac{k_a}{k_d} \cdot [L]}.$$

Multiplying both numerator and denominator by  $K_d \stackrel{\text{def}}{=} k_d/k_a$ , we get

$$\theta = \frac{[L]}{K_d + [L]}.$$

This is exactly Hill's equation for  $n = 1$ .

*Comment.* A reaction  $2L + P \rightarrow L_2P$  can explain  $n = 2$ . Indeed, in this case, the chemical kinetics leads to the following formula for the equilibrium:  $k_a \cdot [L]^2 \cdot [P] = k_d \cdot [L_2P]$ , hence

$$[L_2P] = \frac{k_a}{k_d} \cdot [L]^2 \cdot [P].$$

Here, the ratio  $\theta$  between the concentration  $[L_2P]$  of the bound macromolecule and the total concentration  $[P] + [L_2P]$  of free ( $P$ ) and bound ( $L_2P$ ) macromolecules is equal to

$$\theta = \frac{[L_2P]}{[P] + [L_2P]} = \frac{\frac{k_a}{k_d} \cdot [L]^2 \cdot [P]}{[P] + \frac{k_a}{k_d} \cdot [L]^2 \cdot [P]}.$$

Dividing both numerator and denominator by  $[P] \cdot k_a/k_d$ , and denoting  $K_d = k_d/k_a$ , we get

$$\theta = \frac{[L]^2}{K_d + [L]^2},$$

i.e., Hill's equation for  $n = 2$ .

Similarly, Hill's formula for an arbitrary integer value of  $n$  can be explained if we apply chemical kinetics to the reaction  $nL + P \rightarrow L_nP$ .

**Remaining challenge.** In practice, we often observe non-integer values  $n$ . Such values are difficult to explain by chemical kinetics. As a result, since its invention in 1910, Hill's equation remains a semi-empirical formula.

It is desirable to provide a theoretical explanation for this formula.

## 2 Our Explanation of Hill's Equation

**Towards generalization of chemical kinetics.** In the traditional chemical kinetics, the rate  $r$  of the reaction  $A + B \rightarrow C$  is  $r = \text{const} \cdot [A] \cdot [B]$ . This formula, as we have mentioned, only explains the  $n = 1, 2, \dots$  cases.

To explain the general case of Hill's equation, we need to consider a more general formula  $r = f([A], [B])$ .

**Scale invariance.** Which formulas  $r = f([A], [B])$  should we consider? To answer this question, let us take into account that the numerical value of a quantity depends on the choice of a measuring unit. For example, 2 m is equal to 200 cm.

In particular, if we replace a unit for measuring the concentration  $[A]$  by a  $\lambda_A$  times smaller unit, we get a new numerical value  $[A]' = \lambda_A \cdot [A]$ . Similarly, for  $B$ , we get  $[B]' = \lambda_B \cdot [B]$ . It makes sense to require that the dependence of the reaction rate is the same in the new unit – if we appropriately re-scale the reaction rate  $r$ .

In more precise terms, we assume that for every  $\lambda_A > 0$  and  $\lambda_B > 0$ , there exists a  $\mu$  s.t. if  $r = f([A], [B])$ , then  $\mu(\lambda_A, \lambda_B) \cdot r = f(\lambda_A \cdot [A], \lambda_B \cdot [B])$ . In other words, the dependence  $f([A], [B])$  is such that for every  $\lambda_A$  and  $\lambda_B$ , there exists a  $\mu$  for which: if  $r = f([A], [B])$  then  $r' = f([A]', [B]')$ , where  $[A]' = \lambda_A \cdot [A]$ ,  $[B]' = \lambda_B \cdot [B]$ , and  $r' = \mu \cdot r$ . It is also natural to assume that the dependence  $f([A], [B])$  is differentiable.

**What we can conclude from scale-invariance.** Our assumption means that for all  $x > 0$ ,  $y > 0$ ,  $\lambda_A > 0$ , and  $\lambda_B > 0$ , we have  $f(\lambda_A \cdot x, \lambda_B \cdot y) = \mu(\lambda_A, \lambda_B) \cdot f(x, y)$ .

If  $f(x_0, y_0) = 0$  for some  $x_0 > 0$  and  $y_0 > 0$ , then for every other pairs  $(x, y)$  of positive numbers, we could take  $\lambda_A = x/x_0$  and  $\lambda_B = y/y_0$  and get

$$f(x, y) = f(\lambda_A \cdot x_0, \lambda_B \cdot y_0) = \mu(\lambda_A, \lambda_B) \cdot f(x_0, y_0) = \mu(\lambda_A, \lambda_B) \cdot 0 = 0.$$

Thus, if the function is not identically 0, we conclude that  $f(x, y) \neq 0$  for all  $x > 0$  and  $y > 0$ .

We assumed that the function  $f(x, y)$  is differentiable. Thus, we can conclude that the function

$$\mu(\lambda_A, \lambda_B) = \frac{f(\lambda_A \cdot x, \lambda_B \cdot y)}{f(x, y)}$$

is also differentiable, as a ratio of two differentiable functions.

Let us take  $\lambda_B = 1$ . In this case, the above equation takes the form

$$f(\lambda_A \cdot x, y) = \mu(\lambda_A, 1) \cdot f(x, y).$$

When we differentiate both sides of this equation with respect to  $\lambda_A$ , we get

$$x \cdot f'(\lambda_A \cdot x, y) = \mu'(\lambda_A) \cdot f(x, y),$$

where  $f'$  denotes the partial derivative over  $x$ . Substituting  $\lambda_A = 1$ , we get

$$x \cdot f'(x, y) = \alpha \cdot f(x, y),$$

where we denoted  $\alpha \stackrel{\text{def}}{=} \mu'(1)$ . In other words, we get an equation

$$x \cdot \frac{df}{dx} = \alpha \cdot f.$$

To solve this equation, we separate the variables, i.e., we move all the terms related to  $x$  to one side and all terms related to  $f$  to another side. As a result, we get the following equation:

$$\frac{df}{f} = \alpha \cdot \frac{dx}{x}.$$

Integrating, we get

$$\ln(f(x, y)) = \alpha \cdot \ln(x) + c_1(y),$$

with an integration constant  $c_1$  possibly different for different values  $y$ . From the logarithm, we can now reconstruct the original function as

$$f(x, y) = \exp(\ln(f(x, y))) = \exp(\alpha \cdot \ln(x) + c_1(y)) = \exp(\alpha \cdot \ln(x)) \cdot c_2(y),$$

where we denoted  $c_2(y) \stackrel{\text{def}}{=} \exp(c_1(y))$ . Here

$$\exp(\alpha \cdot \ln(x)) = (\exp(\ln(x)))^\alpha = x^\alpha,$$

so we conclude that

$$f(x, y) = c_2(y) \cdot x^\alpha.$$

If instead we take  $\lambda_A = 1$ , differentiate with respect to  $\lambda_B$  and then take  $\lambda_B = 1$ , we similarly get

$$f(x, y) = c_3(x) \cdot y^\beta$$

for some constant  $\beta$  and for some function  $c_3(x)$ .

The two expressions for  $f(x, y)$  must coincide, so we have

$$c_2(y) \cdot x^\alpha = c_3(x) \cdot y^\beta$$

for all  $x$  and  $y$ . In particular, for  $x = 1$ , we get  $c_2(y) = c_3(1) \cdot y^\beta$ . Substituting this expression for  $c_2(y)$  into the above formula  $f(x, y) = c_2(y) \cdot x^\alpha$ , we conclude that

$$f(x, y) = \text{const} \cdot x^\alpha \cdot y^\beta.$$

So, for the reaction  $A + B \rightarrow C$ , the reaction rate is

$$f([A], [B]) = k_a \cdot [A]^\alpha \cdot [B]^\beta$$

for some constants  $k_a$ ,  $\alpha$ , and  $\beta$ .

A similar analysis can be applied to derive the reaction rate  $f([C])$  for the reaction  $C \rightarrow A + B$ . In this case, scale invariance means that for every  $\lambda_C$ , there exists a  $\mu(\lambda_C)$  for which  $f(\lambda_C \cdot z) = \mu(\lambda_C) \cdot f(z)$ . A similar analysis leads us to the conclusion that  $f(z) = \text{const} \cdot z^\gamma$  for some constant  $\gamma$ .

Thus, for the reaction  $C \rightarrow A + B$ , the the scale-invariant reaction rate is equal to

$$f([C]) = k_d \cdot [C]^\gamma$$

for some constants  $k_d$  and  $\gamma$ .

**Derivation of Hill's equation.** Under the above generalized chemical kinetics equations, the equilibrium is attained when

$$\frac{d[L]}{dt} = -f([L], [P]) + f([LP]) = -k_a \cdot [L]^\alpha \cdot [P]^\beta + k_d \cdot [LP]^\gamma = 0.$$

So, at the equilibrium, we have

$$\frac{k_a}{k_d} \cdot [L]^\alpha \cdot [P]^\beta = [LP]^\gamma$$

and

$$[LP] = C \cdot [L]^n \cdot [P]^{\beta/\gamma},$$

with  $C \stackrel{\text{def}}{=} (k_a/k_d)^{1/\gamma}$  and  $n \stackrel{\text{def}}{=} \alpha/\gamma$ .

When  $\beta = \gamma$ , we have  $[P] + [LP] = (1 + C \cdot [L]^n) \cdot [P]$ , hence

$$\theta = \frac{[LP]}{[P] + [LP]} = \frac{C \cdot [L]^n}{1 + C \cdot [L]^n}.$$

Dividing both numerator and denominator by  $C$ , we get the desired Hill's equation

$$\theta = \frac{[L]^n}{K_d + [L]^n},$$

with  $K_d \stackrel{\text{def}}{=} 1/C$ .

### 3 Conclusions

In biochemistry, the proportion of the bound macromolecules is often described by Hill's equation

$$\theta = \frac{[L]^n}{K_d + [L]^n}.$$

When  $n$  is an integer, this equation can be explained by chemical kinetics, where the rate of the reaction  $A + B \rightarrow C$  is equal to  $k \cdot [A] \cdot [B]$ . However, in practice,  $n$  is often not an integer, and so the chemical kinetics explanation is not applicable.

In this paper, we generalize the traditional equations of chemical kinetics by assuming that the reaction rate  $f([A], [B])$  is scale-invariant but can be more general than the product. As a result, we get a family of formulas that include Hill's equation as a particular case. Thus, we get a theoretical explanation for Hill's equation.

We also get a more general formula that may be useful to explain possible deviation from Hill's equation.

### Acknowledgments

This work was partly supported by the National Science Foundation grant DUE-0926721 "UBM Institutional: Undergraduate Training in Bioinformatics".

The author is greatly thankful to his mentors: Dr. Ming-Ying Leung, Director of the Bioinformatics program, Dr. Mahesh Narayan, Department of Chemistry, and Dr. Vladik Kreinovich, Department of Computer Science, and to all the participants of the 11th NMSU/UTEP Workshop on Mathematics, Computer Science, and Computational Sciences (Las Cruces, New Mexico, March 31, 2012) for valuable suggestions.

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